CHROMOSOME REPORTS IN SOUTH AMERICAN NICOTIANEAE (SOLANACEAE), WITH PARTICULAR REFERENCE TO *NIEREMBERGIA*[1,2]

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ABSTRACT

Chromosome counts and karyotype information from 41 populations of 20 species and 6 varieties of Nicotianeae (Solanaceae) from South America belonging to *Bouchetia* Dunal ($x = 8$), *Fabiana* Ruiz & Pav. ($x = 9$), *Leptoglossis* Benth. ($x = 10$), *Nierembergia* Ruiz & Pav. ($x = 8, 9$), and *Petunia* Juss. ($x = 7, 9$) are given, including first reports from 14 taxa: *F. densa* Rémy, *N. ericoides* Miers, and *P. patagonica* (Speg.) Millán, with $2n = 2x = 18$, and *N. browallioides* Griseb., *N. calycina* Hook., *N. graveolens* A. St. Hil., *N. linariifolia* Graham var. *glabriuscula* (Dunal) A. A. Cocucci & Hunz., var. *pampeana* (Millán) A. A. Cocucci & Hunz., and var. *pinifolioides* (Millán) A. A. Cocucci & Hunz., *N. pulchella* Miers var. *pulchella* and var. *macrocalyx* (Millán) A. A. Cocucci & Hunz., *N. rivularis* Miers, *N. tucumanensis* Millán, and *N. veitchii* Hook., with $2n = 2x = 16$. All species studied are diploids, and most have $2n = 16$. In addition, infraspecific polyploidy is recorded for the first time in *N. rigida* Miers ($2n = 4x = 32$) and confirmed in *N. aristata* D. Don ($n = 3x = 24$, $2n = 6x = 48$). In general, karyotypes of the examined species are symmetrical and composed of comparatively small and uniformly sized chromosomes, mostly of the metacentric (*m*) type except in *L. linifolia* (Miers) Griseb., *P. axillaris* (Lam.) Britton, Stern & Poggenb., and *P. patagonica*, where submetacentric (*sm*) chromosomes are predominant. There are one or, rarely, two chromosomes bearing nucleolar organizing regions per basic complement. In *Nierembergia*, karyotype data reflect species grouping. Results suggest that *Bouchetia* is the closest taxon to *Nierembergia*, while *Leptoglossis*, *Petunia*, and *Fabiana* appear more distant. Possible chromosome number evolution in the tribe is speculated based on molecular phylogenetic studies by other authors. In *Nierembergia*, $x = 9$ is proposed to be derived from $x = 8$. Dysploid changes in chromosome number and conservation of chromosome morphology in the karyotypes appear as important evolutionary events in the tribe, while polyploidy is noteworthy only in *Nicotiana* L.

Key words: *Bouchetia*, chromosome numbers, evolution, *Fabiana*, karyotypes, *Leptoglossis*, *Nierembergia*, *Petunia*, polyploidy, Solanaceae, systematics.

The tribe Nicotianeae G. Don (Cestroideae Schltdl., Solanaceae) is composed of plants native to the New World, principally in South America, with *Nicotiana* L. also extending to other continents. As recently proposed by Hunziker (2001), three subtribes are recognized: Nicotianinae Hunz., comprising *Nicotiana* with 67 species (77 after Chase et al., 2003), *Petunia* Juss. with around 34 species (over 40 following Mishiba et al., 2000), and *Fabiana* Ruiz & Pav. (15 species); Nierembergiinae Hunz. & A. A. Cocucci, including *Nierembergia* Ruiz & Pav. (21) and *Bouchetia* Dunal (3); and Leptoglossinae Hunz., encompassing *Leptoglossis* Benth. (7), *Hunzikeria* D'Arcy (3), and *Plowmania* Hunz. & Subils (1, *P. nyctaginoides* (Standl.) Hunz. & Subils). Although much cytogenetic work has been carried out in *Nicotiana* (Goodspeed, 1954; Japan Tobacco, 1994; Chase et al., 2003) and *Petunia* (Wijsman et al., 1983; Maizonnier, 1984; Mishiba et al., 2000), the remaining genera of Nicotianeae have been poorly studied karyologically (see Table 1).

The chromosome number and karyotype information of 20 South American species belonging to five genera of Nicotianeae, i.e., *Bouchetia*, *Fabiana*, *Leptoglossis*, *Petunia*, and *Nierembergia*, are reported herein as a part of a broad cytogenetic study. Our work in *Nierembergia* and related taxa has the following objectives: (1) to report original counts and karyotype analyses for karyologically unknown taxa in Solanaceae, (2) to increase our knowledge of the systematic relationships in the tribe Nicotianeae, and (3) to gain a better insight into chromosomal variation and

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Table 1. Previously published chromosome counts in Nicotianeae (Solanaceae), excluding *Nicotiana* and *Petunia*. Valid names for *Nierembergia* species according to Cocucci & Hunziker (1995). Synonymous names under which chromosome counts were originally published are indicated in brackets.

Taxon	*n*	2*n*	Reference
Hunzikeria texana (Torr.) D'Arcy [sub nom. *Leptoglossis texana* (Torr.) A. Gray]	16		Whalen (1979)
Bouchetia anomala (Miers) Britton & Rusby	8		Di Fulvio (1978)
Bouchetia erecta Dunal	8		Whalen (1979)
Fabiana denudata Miers	9		Moscone (1992)
Fabiana imbricata Ruiz & Pav.	9		Desai (1970)
		18	Goodspeed (1933)
Leptoglossis linifolia (Miers) Griseb.	10		Subils (1979)
Leptoglossis schwenckioides Benth. [sub nom. *Salpiglossis schwenckioides* (Benth.) Wettstein]		20	Diers (1961)
Nierembergia aristata D. Don [sub nom. *N. stricta* Miers]	8		Di Fulvio (1976a)
	24		Di Fulvio (1984)
Nierembergia linariifolia Graham var. *linariifolia* [sub nom. *N. hippomanica* Miers]	8		Di Fulvio (1976a)
	9		Ratera (1952)
		16	Di Fulvio (1976a)
Nierembergia repens Ruiz & Pav.		16	Shizukawa & Mii (1997)
Nierembergia rigida Miers [sub nom. *N. aristata* Sweet]	8		Di Fulvio (1978)
	9		Ratera (1969)
Nierembergia scoparia Sendtn. [sub nom. *N. frutescens* Durieu]		18	Goodspeed (1933)

possible karyo-evolutionary trends, especially in the subtribe Nierembergiinae.

Bouchetia is a small genus of hemi-cryptophytes displaying a disjunct distribution, with *B. anomala* (Miers) Britton & Rusby in southern Brazil, Paraguay, Uruguay and north to central Argentina, and two other species in North America (Hunziker, 2001). On the other hand, *Fabiana* is a western South American endemic taxon of microphyllous and resiniferous shrubs or chamaephytes distributed along the Andes from southern Peru to southern Chile and Argentina, which reaches lowland regions in Patagonia. Considering the shrubby species examined here, *F. densa* Rémy inhabits southern Bolivia and northwestern Argentina, *F. denudata* Miers extends from northern Chile through northern and central Andean areas of Argentina reaching northern Patagonia, and *F. imbricata* Ruiz & Pav. grows in the central and southern Andean regions of Argentina and Chile (Barboza & Hunziker, 1993).

Leptoglossis comprises xerophytic species inhabiting Peru except for the one examined here, the perennial herb *L. linifolia* (Miers) Griseb., endemic to the central and western Argentine lowlands (Hunziker & Subils, 1979). *Petunia* is a South American genus, with one species also in Central and North America. Concerning the two entities here analyzed, *P. axillaris* (Lam.) Britton, Stern & Poggenb. is an annual herb growing in southern Brazil, Bolivia, Uruguay, and Argentina and *P. patagonica* (Speg.) Millán is a chamaephyte endemic to the Argentine province of Santa Cruz (Figueroa Romero, 1999; Hunziker, 2001).

Finally, *Nierembergia*, focused on in this contribution, is South American (except for one species in Mexico), with its principal distribution in the western and southern regions, and the center of diversification in Argentina where 15 species are found (Hunziker, 2001). Its members have nectarless flowers and, in most cases, are unique in the family for producing oils that attract oil-collecting bees (Cocucci, 1991). Several species have become noteworthy as poisonous weeds toxic to cattle, valuable ornamentals, useful plants for genetic engineering, or for potential pharmacological uses with respect to cardenolides and their antitumoral activity (Gil et al., 1995; Godo et al., 1997; Hunziker, 2001).

Species presently studied include all life forms known in *Nierembergia*; they grow in distinct habitats and may have either a widespread or restricted geographical distribution. Some are small shrubs that grow mainly in mountain regions, i.e., *N. browallioides* Griseb., *N. ericoides* Miers, *N. linariifolia* Graham var. *glabriuscula* (Dunal) A. A. Cocucci & Hunz. and var. *pinifolioides* (Millán) A. A. Cocucci & Hunz., *N. pulchella* Miers, and *N. tucumanensis* Millán, or in lowlands, i.e., *N. graveolens* A. St.-Hil., *N. linariifolia* var. *linariifolia* and var. *pampeana* (Millán) A. A. Cocucci & Hunz., and *N. scoparia* Sendtn. Others are rhizomatous, stoloniferous, or tuber-bearing, prostrate (rarely ascendent) herbs from meadows and marshes, i.e., *N. aristata* D. Don, *N. calycina* Hook., *N. repens* Ruiz & Pav., *N. rigida* Miers, *N. rivularis* Miers, and *N. veitchii* Hook. *Nierembergia rigida* and *N. linariifolia* exhibit the broadest geographical distribution, extending from southern Brazil to southern Argentina, reaching Patagonia, while *N. tucumanensis*, *N. veitchii*, *N. browallioides*, and *N. pulchella* are mainly distributed in northwestern Argentina, the last two species also found in southern Bolivia. *Nierembergia aristata*, *N. graveolens*, *N. scoparia*, and *N. rivularis* inhabit southern Brazil, Uruguay, and northeastern Argentina, with the first species reaching Paraguay and the last one into Bolivia. Lastly, *N. calycina*, *N. ericoides*, and *N. repens* are endemic to eastern Argentina and Uruguay, Argentina (Buenos Aires province), and southern Chile, respectively (Millán, 1941; Cosa de Gastiazoro, 1989; Cocucci & Hunziker, 1995).

MATERIALS AND METHODS

The provenance of plant material studied is presented in Table 2. The respective voucher specimens were deposited in the herbarium of Museo Botánico de Córdoba, Argentina (CORD).

Somatic chromosomes were observed in squashed root meristems obtained from seed germination. The root apices were fixed in 1:3 acetic acid/ethanol mixture for 12 hours (h) after a pretreatment either in paradichlorobenzene-saturated solution for 2 h at room temperature or in 2 mM 8-hydroxyquinoline for 8 h at 8°C. In most cases, root tips were then stained according to Feulgen's technique by using Schiff's reagent for 1 ½ h in darkness, after hydrolysis in 5 N hydrochloric acid for 50 min at room temperature (Jong, 1997). In some cases, meristems were stained with alcoholic hydrochloric acid-carmine (Snow, 1963) for 3–4 days at room temperature. Meristem cells were isolated, macerated, and squashed in a drop of 2% aceto-carmine after the first procedure or 45% acetic acid after the second staining method. Comparisons of metaphase chromosome size on test preparations after the different pretreatment and staining conditions demonstrated no significant differences between the procedures. Meiotic chromosomes were examined in pollen mother cells from squashed young anthers fixed as described for root apices and stained with aceto-carmine. After staining, slides were made permanent by freezing with liquid CO_2 (Bowen, 1956), removing the coverslip and mounting in Euparal (Chroma, Germany), or without removing the coverslip (Bradley, 1948) and mounting in Rhenohistol (Merck, Darmstadt).

Somatic and meiotic chromosomes were observed and photographed with a Leica DMLB microscope equipped with a Leica DC 250 digital camera and the Leica IM 1000 image management system. Chromosome numbers were determined from 14 to 177 cells of one to 65 seedlings of each species and variety; all counts were made from somatic metaphases, except for the accession *Acosta et al. 32* (CORD), which was analyzed in meiosis (Table 2). Somatic chromosomes were measured and classified according to their arm ratio (r = long arm/short arm length) as recognized after Levan et al. (1964) with the modifications introduced by Schlarbaum and Tsuchiya (1984) as: *m*—metacentric (r = 1.00–1.29), *msm*—meta-submetacentric (r = 1.30–1.69), *sm*—submetacentric (r = 1.70–2.99), *st*—subtelocentric (r = 3.00–6.99), and *t*—telocentric (r = 7.00 and higher).

Leaf and calyx measurements were made from voucher specimens of *N. aristata* and *N. rigida* (Table 3). The Mann-Whitney U-test to at least the 0.01 significance level was used to test for significant differences in the median values between vouchers of the same species with different ploidy level (Sokal & Rohlf, 1995). Statistical analysis was performed by using INFOSTAT, version 1.1 (Infostat Group, 2002).

RESULTS

Chromosome counts from 41 populations of 20 South American species of Nicotianeae (Solanaceae) belonging to *Bouchetia* (1 species), *Fabiana* (3), *Leptoglossis* (1), *Petunia* (2), and *Nierembergia* (13 species, 6 varieties) are reported (Table 2). The somatic chromosome number $2n = 2x = 20$ is found in *Leptoglossis linifolia* (Fig. 1A), while two other figures are observed in *Petunia*, $2n = 2x = 14$ in *P. axillaris* (Fig. 1B) and $2n = 2x = 18$ in *P. patagonica* (Fig. 1C). In addition, $2n = 2x = 18$ is also present in the examined species of *Fabiana*, i.e., *F. densa* (Fig. 1D), *F. denudata* (Fig. 1E), and *F. imbricata* (Fig. 1F), and in two species of *Nierembergia*, *N. ericoides* (Fig. 1H) and *N. scoparia* (Fig. 1I). On the other hand, $2n = 2x = 16$ is recorded in *Bouchetia anomala* (Fig. 1L) and the remaining species of *Nierembergia* studied, i.e., *N. aristata*, *N. browallioides* (Fig. 1G), *N. calycina* (Fig. 2A), *N. graveolens* (Fig. 2D), *N. linariifolia* (Fig. 2E), *N. pulchella* (Fig. 2 B–C), *N. repens* (Fig. 2F), *N. rivularis* (Fig. 2G), *N. rigida*, *N. tucumanensis* (Fig. 2H), and *N. veitchii* (Fig. 2I). In addition, two populations of *N. aristata* display $2n = 6x = 48$ (Fig. 1K), and one sample of *N. rigida* exhibits $2n = 4x = 32$ (Fig. 1J).

The chromosomes are small, ranging from 1.52 to 3.52 μm. The average chromosome length (standard deviation) is 2.54 (0.27) μm in *Bouchetia anomala* and 2.33 (0.24) μm in *Leptoglossis linifolia*, and, in genera where more than one species were examined, it ranges from 2.30 (0.26)–2.90 (0.38) µm in *Fabiana*, 2.87 (0.30)–3.01 (0.21) µm in *Petunia* (the higher value in *P. patagonica*), and 2.17 (0.23)–2.87 (0.30) µm in *Nierembergia*. Species of the latter genus can be separated into two groups according to chromosome size. On the one hand, *N. browallioides*, *N. linariifolia*, *N. pulchella*, and *N. tucumanensis* all have larger chromosomes, with mean chromosome length values above 2.70 µm. On the other hand, the remaining species display smaller chromosomes, with mean estimates below 2.40 µm, except for *N. calycina* (2.59 µm) and *N. ericoides* (2.61 µm).

Species studied have one chromosome pair carrying nucleolar organizing regions (NORs) plus attached satellites of variable size (e.g., Fig. 1B–E, G–H), except *Bouchetia anomala* (Fig. 1L), *Nierembergia graveolens* (Fig. 2D), *N. linariifolia* var. *glabriuscula*, *N. repens*, and the accession *Di Fulvio 847* (CORD) of *N. aristata*, where two of such pairs were observed. In the examined sample of *L. linifolia* no NORs could be detected (Fig. 1A). Usually, NORs are visible in both members of the respective chromosome pairs, although in several metaphases of each sample they were missing in one homologue (Figs. 1F, I, K; 2F). NORs appear on short arms, except in *N. calycina*, where they were seen on long arms (Fig. 2A).

In general, the Nicotianeae studied have karyotypes with chromosomes that are homogeneous in size and predominantly of the *m* type. The respective karyotype formulae are given in Table 2. Particularly, in *Nierembergia*, *N. browallioides*, *N. calycina*, *N. tucumanensis*, and the varieties of *N. pulchella* (2) and *N. linariifolia* (4) examined, only *m* chromosomes or a majority of *m* and one to two *msm* chromosomes were observed. In contrast, *N. graveolens*, *N. repens*, *N. rigida*, *N. rivularis*, and *N. veitchii* possessed *sm* (1–2) in addition to *msm* (1–2) pairs, while *N. aristata* showed three *msm* pairs. *Leptoglossis linifolia* (7 *sm* pairs), *P. axillaris* (3 *sm* and 1 *msm* pairs), and *P. patagonica* (4 and 1), are distinct in having a minority of *m* chromosomes. *Bouchetia anomala*, the three *Fabiana* species, *N. ericoides*, and *N. scoparia* were the only taxa observed with one *st* pair (in addition to 1 *sm* and/or 1 *msm* pairs), which, in the last two species, was the smallest pair at half the length of the largest pair of the chromosomal complement (Fig. 1H–I).

Statistical comparison of leaf and calyx size yields significant differences between $2n = 2x = 16$ and $2n = 6x = 48$ accessions of *Nierembergia aristata* and between $2n = 2x = 16$ and $2n = 4x = 32$ accessions of *N. rigida*, except for the calyx length in the first species, with polyploids exhibiting greater dimensions for these structures than diploids (Table 3). In *N. aristata*, all individuals examined of the accession *Acosta et al. 32* ($2n = 6x = 48$) showed multivalents in diakinesis-metaphase I, with the following configurations being the most frequent: 2 VI + 1 IV + 16 II (27% of the cells) and 2 VI + 18 II (13%) (data not shown).

DISCUSSION

Chromosome counts from 41 populations of 20 species and 6 varieties of Nicotianeae from South America belonging to *Bouchetia*, *Fabiana*, *Leptoglossis*, *Nierembergia*, and *Petunia* are given, including first reports from 14 taxa—*Fabiana densa*, *Nierembergia browallioides*, *N. calycina*, *N. ericoides*, *N. graveolens*, three out of the four varieties of *N. linariifolia* (vars. *glabriuscula*, *pampeana*, and *pinifolioides*), two out of the three varieties of *N. pulchella* (vars. *pulchella* and *macrocalyx* (Millán) A. A. Cocucci & Hunz.), *N. rivularis*, *N. tucumanensis*, *N. veitchii*, and *Petunia patagonica*—and a new chromosome number from *N. rigida* (see Table 2).

Chromosome figures obtained in *Bouchetia anomala*, *Fabiana denudata*, *F. imbricata*, *Leptoglossis linifolia*, *Nierembergia aristata*, *N. repens*, and *N. scoparia* agree with previously published reports, most of these made during meiosis (see Table 1). Furthermore, our findings of $2n = 16$ in five populations of *N. linariifolia* var. *linariifolia* and one of *N. rigida* are in accordance with previous counts by Di Fulvio (1976a, 1978) and suggest that $n = 9$, as cited for both species by Ratera (1952, 1969), is unlikely. Finally, the chromosome number observed in *Petunia axillaris* matches previous records both in meiotic (Ferguson, 1924; Ratera, 1952; Moscone, 1992; Stehmann et al., 1997) and mitotic divisions (Steere, 1932; Sullivan, 1947; Rangaswamy & Shivanna, 1967; Sink & Power, 1978; Watanabe et al., 1996; Badr et al., 1997).

BASIC CHROMOSOME NUMBERS

Results confirm the basic chromosome numbers already known for the examined genera, i.e., *Bouchetia* ($x = 8$), *Fabiana* ($x = 9$), *Leptoglossis* ($x = 10$), *Nierembergia* ($x = 8, 9$), and *Petunia* ($x = 7, 9$) (Hunziker, 2001). According to the available chromosome information, Nicotianeae exhibits a dysploid series from $x = 7$ to 12 and some other derived basic numbers, which as arranged in decreasing frequency are: $x = 12, 9, 8, 7, 10, 23, 19$, and 11 (Moscone, 1992; Stehmann et al., 1997; Mishiba et al., 2000; Hunziker, 2001; Chase et al., 2003). All of these numbers are present in *Nicotiana*, where $x = 12$ is the most frequent, as it is the basic number of subgenera *Rustica* (G. Don) Goodsp. and *Tabacum* (G. Don) Goodsp., as well as of seven of the nine sections of

Table 2. List of the taxa and samples studied (all accessions from Argentina unless indicated otherwise), provenance, voucher number (all voucher specimens deposited at CORD), number of seedlings and somatic metaphases (diakinesis-metaphases I in *Acosta et al. 32*) analyzed per sample, somatic chromosome number and karyotype formula. Abbreviations of collectors' name: MCA = M. C. Acosta, JAA = J. A. Ambrosetti, GEB = G. E. Barboza, AC = A. Cicarelli, AAC = A. A. Cocucci, CC = C. Costa, AD = A. Dafni, EDF = E. Di Fulvio, LAD = L. A. Díaz, FE = F. Ehrendorfer, LG = L. Galetto, ATH = A. T. Hunziker, CAK = C. A. Kirkwood, YK = Y. Kurakami, MMe = M. Medina, MMo = M. Moré, EAM = E. A. Moscone, JN = J. Nattero, SS = S. Schneckenburger, ANS = A. N. Sérsic, RS = R. Subils, MT = M. Tomoya, SV = S. Vogel. [^] In parentheses is indicated the number of individuals and metaphases utilized for chromosome measurements per species and variety. *m*, metacentric; *msm*, meta-submetacentric; *sm*, submetacentric; *st*, subtelocentric chromosome. [+] Haploid count in meiosis (diakinesis-metaphase I). * First chromosome report; ** New chromosome number.

Taxon	Provenance and voucher number	No. of seedlings and somatic metaphases analyzed per sample	2*n*	Karyotype formula[^]
Bouchetia anomala (Miers) Britton & Rusby	Prov. Córdoba, Dept. San Justo, betw. Villa Concepción del Tío & Frontera Sur, *EDF 833*.	1, 3	16	6 *m* + 1 *sm* + 1 *st* (5, 6)
B. anomala	Prov. Córdoba, Dept. San Justo, Marull, *EDF 840*.	30, 64	16	6 *m* + 1 *sm* + 1 *st*
B. anomala	Prov. Córdoba, Dept. Punilla, El Durazno, *AAC, ANS & EAM 941*.	34, 110	16	6 *m* + 1 *sm* + 1 st
Fabiana densa Rémy*	Prov. Tucumán, Dept. Tafí del Valle, El Molle, *ATH, GEB & EAM 24884*.	12, 28	18	7 *m* + 1 *sm* + 1 *st* (3, 6)
Fabiana denudata Miers	Prov. Mendoza, Dept. Malargüe, Sierra de Palauco, *JAA, AC & EAM 1417*.	12, 39	18	7 *m* + 1 *sm* + 1 *st* (5, 10)
Fabiana imbricata Ruiz & Pav.	Prov. Chubut, Dept. Futaleufú, Mt. El Dedal, *AAC & SS 404*.	4, 18	18	7 *m* + 1 *sm* + 1 *st* (1, 5)
Leptoglossis linifolia (Miers) Griseb.	Prov. Santiago del Estero, Dept. Ojo de Agua, betw. Loreto & Villa Ojo de Agua, *AAC & ANS 1112*.	12, 33	20	3 *m* + 7 *sm* (3, 5)
Nierembergia aristata D. Don	Prov. Córdoba, Dept. San Justo, betw. Villa Concepción del Tío & Frontera Sur, *EDF 847*.	11, 61	16	5 *m* + 3 *msm* (4, 10)
N. aristata	Prov. Santa Fe, Dept. Capital, betw. La Guardia & Colastiné Norte, *RS, AAC & GEB 1215*.	22, 33	48	?
N. aristata	Prov. Córdoba, Dept. Punilla, San Roque, *MCA, LAD & CAK 32*[+].	2, 5[+]	48	?
Nierembergia browallioides Griseb.*	Prov. Tucumán, Dept. Tafí del Valle, betw. La Bolsa & El Infiernillo, *LG, AAC, ANS & SV 159*.	25, 81	16	6 *m* + 2 *msm* (6, 10)
Nierembergia calycina Hook.*	URUGUAY. Dept. Paysandú, Paysandú, *AAC & ANS 1095*.	8, 43	16	6 *m* + 2 *msm* (3, 5)
Nierembergia ericoides Miers*	Prov. Buenos Aires, Dist. Tandil, Tandil, *AAC & ANS 923*.	9, 21	18	7 *m* + 1 *msm* + 1 *st* (4, 6)
*N. ericoides**	URUGUAY. Dept. Maldonado, Pan de Azúcar, *AAC 1104*.	3, 5	18	7 *m* + 1 *msm* + 1 *st*
*N. ericoides**	URUGUAY. Dept. Lavalleja, Sierras de Solís, *AAC 1106*.	2, 4	18	7 *m* + 1 *msm* + 1 *st*
*N. ericoides**	Prov. Buenos Aires, Dist. Tandil, Tandil, *AAC & ANS 1330*.	10, 31	18	7 *m* + 1 *msm* + 1 *st*
Nierembergia graveolens A. St.-Hil.*	Prov. Entre Ríos, Dept. Federación, Chajarí, *AAC, ANS, MMo, JN & AD 3021*.	1, 14	16	6 *m* + 1 *msm* + 1 *sm* (1, 5)
Nierembergia linariifolia Graham var. *linariifolia*	Prov. Córdoba, Dept. Río Primero, betw. Río Primero & Santa Rosa de Río Primero, *EDF 842*.	37, 85	16	6 *m* + 2 *msm* (8, 10)
N. linariifolia var. *linariifolia*	Prov. Córdoba, Dept. Punilla, Santa María de Punilla, *EAM 162*.	7, 10	16	6 *m* + 2 *msm*

Table 2. Continued.

Taxon	Provenance and voucher number	No. of seedlings and somatic metaphases analyzed per sample	$2n$	Karyotype formula^
N. linariifolia var. *linariifolia*	Prov. Córdoba, Dept. Capital, El Infiernillo, *AAC, ANS & EAM 944.*	1, 3	16	6 *m* + 2 *msm*
N. linariifolia var. *linariifolia*	Prov. Tucumán, Dept. Tafí Viejo, betw. La Sala & Raco, *AAC & ANS 1114.*	1, 4	16	6 *m* + 2 *msm*
N. linariifolia var. *linariifolia*	Prov. Santiago del Estero, Dept. Ojo de Agua, betw. Loreto & Villa Ojo de Agua, *AAC & ANS 1205.*	4, 24	16	6 *m* + 2 *msm*
N. linariifolia var. *glabriuscula* (Dunal) A. A. Cocucci & Hunz.*	Prov. Córdoba, Dept. Punilla, La Posta, *AAC, ANS, FE & EAM 1010.*	2, 5	16	7 *m* + 1 *msm* (3, 6)
N. linariifolia var. *glabriuscula**	Prov. Córdoba, Dept. Punilla, Dos Puertas, *LAD 4.*	9, 39	16	7 *m* + 1 *msm*
N. linariifolia var. *pampeana* (Millán) A. A. Cocucci & Hunz.*	Prov. Río Negro, Dept. Pichi Mahuida, Río Colorado, *AAC & ANS 1265.*	5, 20	16	7 *m* + 1 *msm* (3, 5)
N. linariifolia var. *pinifolioides* (Millán) A. A. Cocucci & Hunz.*	Prov. Córdoba, Dept. Ischilín, betw. Dean Funes & Sauce Punco, *AAC & LAD 1200.*	2, 4	16	6 *m* + 2 *msm* (5, 10)
N. linariifolia var. *pinifolioides**	Prov. Córdoba, Dept. Ischilín, betw. Dean Funes & Tulumba, *AAC & ANS 1318.*	3, 7	16	6 *m* + 2 *msm*
N. linariifolia var. *pinifolioides**	Prov. Córdoba, Dept. Ischilín, Dean Funes, *MCA & LAD 13.*	2, 3	16	6 *m* + 2 *msm*
N. linariifolia var. *pinifolioides**	Prov. Córdoba, Dept. Punilla, La Toma, *AAC 1455.*	6, 43	16	6 *m* + 2 *msm*
N. linariifolia var. *pinifolioides**	Prov. Córdoba, Dept. Punilla, betw. Capilla del Monte & Ongamira, *AAC 1456.*	1, 3	16	6 *m* + 2 *msm*
Nierembergia pulchella Miers var. *pulchella**	Prov. Mendoza, Dept. Las Heras, Villavicencio, *AAC, ANS & CC 1122.*	8, 91	16	7 *m* + 1 *msm* (4, 6)
Nierembergia pulchella var. *macrocalyx* (Millán) A. A. Cocucci & Hunz.*	Prov. La Rioja, Dept. Famatina, Mt. Famatina, *AAC & ANS 972*, leg. MMe.	7, 36	16	8 *m* (4, 6)
Nierembergia repens Ruiz & Pav.	CHILE. Region VIII, Prov. Concepción, betw. Lota & Concepción, *AAC & ANS 961.*	10, 31	16	6 *m* + 1 *msm* + 1 *sm* (4, 5)
Nierembergia rigida Miers	Prov. Córdoba, Dept. San Justo, Marull, *EDF 834.*	29, 53	16	5 *m* + 1 *msm* + 2 *sm* (2, 6)
N. rigida	Prov. Córdoba, Dept. Punilla, Santa María de Punilla, *AAC, ANS & EAM 943***.	8, 58	32	?
Nierembergia rivularis Miers*	URUGUAY. Dept. Soriano, Villa Soriano, *AAC & ANS 1099.*	1, 19	16	5 *m* + 1 *msm* + 2 *sm* (1, 5)
Nierembergia scoparia Sendtn.	URUGUAY. Dept. Paysandú, Tres Bocas, *AAC & ANS 1097.*	6, 36	18	6 *m* + 1 *msm* + 1 *sm* + 1 *st* (3, 5)
Nierembergia tucumanensis Millán*	Prov. Tucumán, Dept. Tafí del Valle, betw. El Infiernillo & Amaichá del Valle, *AAC, YK & MT 1451.*	4, 17	16	7 *m* + 1 *msm* (3, 7)
Nierembergia veitchii Hook.*	Prov. Tucumán, Dept. Tafí Viejo, San Javier, *AAC & ANS 1113.*	7, 24	16	5 *m* + 2 *msm* + 1 *sm* (3, 5)
Petunia axillaris (Lam.) Britton, Stern & Poggenb.	Prov. Córdoba, Dept. Calamuchita, Falda del Sauce, *EAM 189.*	6, 32	14	3 *m* + 1 *msm* + 3 *sm* (3, 6)
Petunia patagonica (Speg.) Millán*	Prov. Santa Cruz, Dept. Lago Argentino, Estancia La Victoria, *AAC & ANS 471.*	26, 125	18	4 *m* + 1 *msm* + 4 *sm* (5, 6)

Table 3. Comparison of leaf and calyx size between populations of different ploidy level in *Nierembergia aristata* and *N. rigida*. All measurements in mm. Range, mean (standard deviation), median, and sample size (n) are given for each variable. ** Median values differ significantly between vouchers of the same species with different ploidy level (p minor to 0.01). Voucher specimens of accessions: *N. aristata* 2*x*, *Di Fulvio 847*, and 6*x*, *Subils et al. 4215*; *N. rigida* 2*x*, *Di Fulvio 834*, and 4*x*, *Cocucci et al. 943*.

		Leaf size		Calyx size	
Species	Ploidy level	Length	Width	Length	Calyx lobe width
Nierembergia aristata	2*x*	9.00–30.00 16.32 (5.90) 14.50** n = 50	0.50–2.00 1.24 (0.36) 1.20** n = 50	8.00–15.00 11.33 (2.12) 12.00 n.s. n = 21	0.50–1.20 0.78 (0.18) 0.70** n = 21
N. aristata	6*x*	11.50–34.00 21.83 (4.91) 21.45** n = 50	1.20–4.50 2.49 (0.91) 2.10** n = 50	10.00–16.00 12.32 (1.48) 12.00 n.s. n = 25	0.70–1.80 1.10 (0.25) 1.00** n = 25
Nierembergia rigida	2*x*	6.00–24.00 14.95 (4.28) 15.00** n = 50	0.50–0.90 0.59 (0.10) 0.60** n = 50	7.00–22.00 13.24 (3.79) 13.00** n = 25	0.60–1.50 0.90 (0.25) 0.80** n = 25
N. rigida	4*x*	8.00–37.00 18.67 (7.28) 18.25** n = 50	0.50–1.20 0.75 (0.19) 0.75** n = 50	12.50–23.50 15.98 (2.97) 15.00** n = 25	0.70–2.00 1.25 (0.29) 1.20** n = 25

subgenus *Petunioides* (G. Don) Goodsp.; however, $x = 12$ is absent in the remaining genera of the tribe. In fact, $x = 12$ is by far the most common basic number in the whole family where it is recorded in more than 50% of the species karyologically examined. Although typical in subfamily Solanoideae, this figure is rare in subfamily Cestroideae, with the exception of *Nicotiana*, as it has been cited just in one species of *Schwenckia* L. and in the monotypic *Streptosolen* Miers.

Both $x = 9$ and $x = 8$ appear in four out of the eight genera of Nicotianeae; thus, they are the basic chromosome numbers most widely distributed in the tribe. The former number is found in *Nicotiana* sect. *Alatae* Goodsp. (five of nine species) and section *Suaveolentes* Goodsp. (two species), *Fabiana*, *Nierembergia* (two species), and *Petunia*. In the latter genus, $x = 9$ is the most common number, which appears in those species transferred to *Calibrachoa* La Llave & Lex. by Wijsman (1990) and some of *Petunia* sensu Jussieu (1803). On the other hand, $x = 8$ is the only basic number reported for *Bouchetia* and *Hunzikeria*, the typical number of *Nierembergia*, and one of the numbers of *Nicotiana* sect. *Suaveolentes*, where the complete series is present. With respect to $x = 7$, this is documented in all species of *Petunia* sensu Wijsman (1990) and a few species of *Nicotiana* sect. *Suaveolentes*. Finally, $x = 10$ appears only in *Leptoglossis*, several species of *Nicotiana* sect. *Suaveolentes*, and two of section *Alatae*. It should be noted that the basic chromosome numbers found by us, with the exception of $x = 7$, are also cited in other tribes within subfamily Cestroideae, i.e., $x = 8$ in Cestreae G. Don (*Cestrum* L. and *Vestia* Willd.), $x = 9$ in Latueae Hunz. & Barboza (*Latua pubiflora* (Griseb.) Baill.), and $x = 10$ in Browallieae Hunz. (*Browallia* L.) and Schwenckieae Hunz. (*Schwenckia americana* L.) (Hunziker, 2001; Chiarini, 2003).

For evolutionary considerations, a comprehensive scheme of possible chromosome number changes during speciation in the Solanaceae is still lacking, and even its original basic number is a matter to be clarified. In this sense, Raven (1975) has speculated that $x = 7$ could be the original number for Solanales, while $x = 12$ may have derived from the tetraploid level ($n = 14$) by aneuploid reduction in the early history of Solanaceae, subsequently giving rise to the lower numbers of the series in Nicotianeae by the same mechanism, ending in $x = 7$. Nevertheless, chloroplast DNA sequence analyses support the ancestral placement of Cestroideae sensu D'Arcy (1991) in the family and the apomorphic condition of $x = 12$ (Olmstead & Palmer, 1992; Olmstead et al., 1999).

In the phylogenetic trees presented by Olmstead & Palmer (1992) and Olmstead et al. (1999), where a broad sample of Solanaceae genera was included, one may speculate a hypothetical direction of

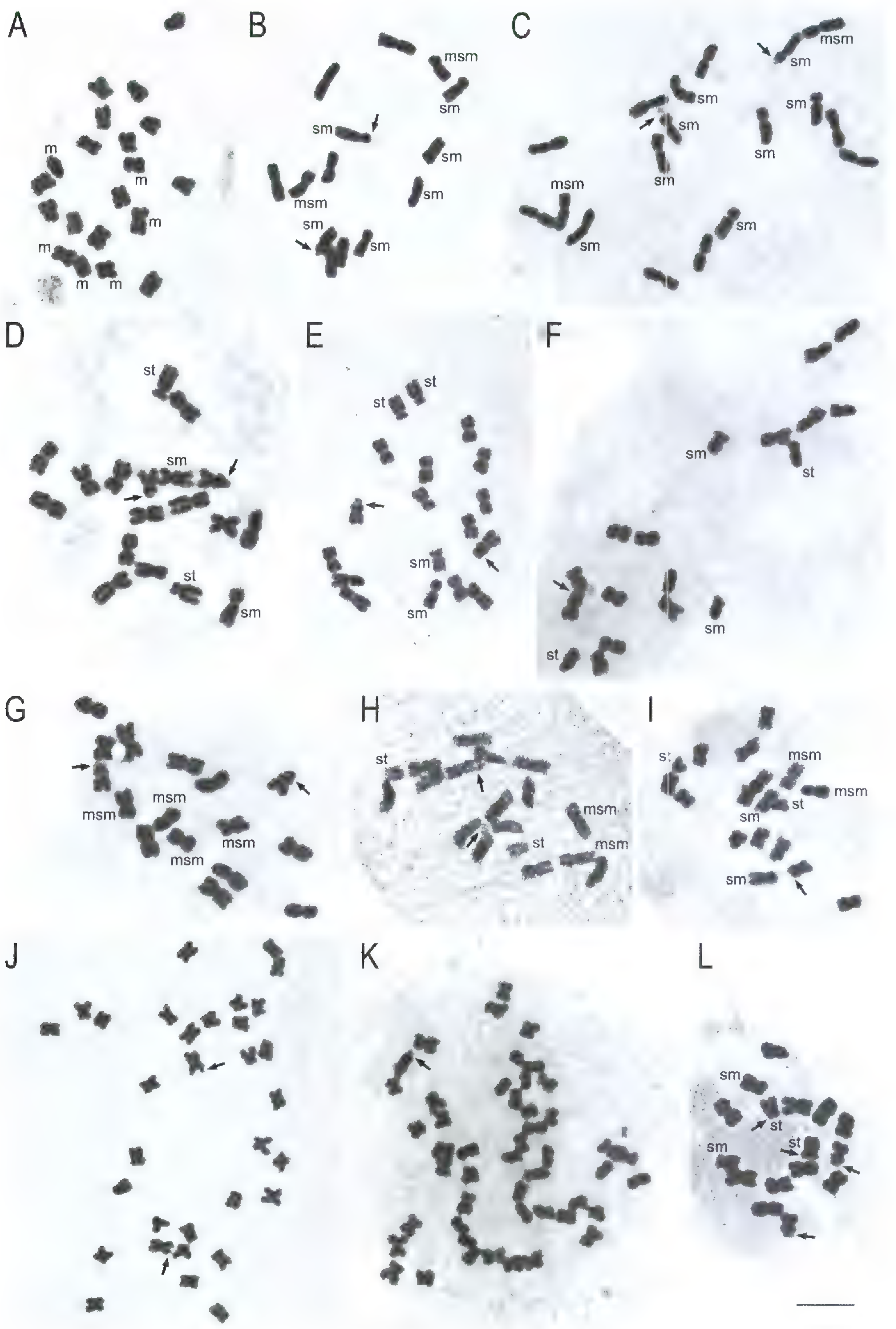

Figure 1. Somatic metaphases of *Leptoglossis*, *Petunia*, *Fabiana*, *Nierembergia*, and *Bouchetia* species. —A. *L. linifolia* (2*n* = 20). —B. *P. axillaris* (2*n* = 14). —C. *P. patagonica* (2*n* = 18). —D. *F. densa* (2*n* = 18). —E. *F. denudata* (2*n* = 18). —F. *F. imbricata* (2*n* = 18). —G. *N. browallioides* (2*n* = 16). —H. *N. ericoides* (2*n* = 18). —I. *N. scoparia* (2*n* = 18). —J. *N. rigida* (2*n* = 32). —K. *N. aristata* (2*n* = 48). —L. *B. anomala* (2*n* = 16). Arrows (↑) indicate nucleolar organizing regions (NORs). Less frequent chromosome types are indicated: *m*, metacentric; *msm*, meta-submetacentric; *sm*, submetacentric; *st*, subtelocentric. Scale bar = 5 μm is the same for all figures.

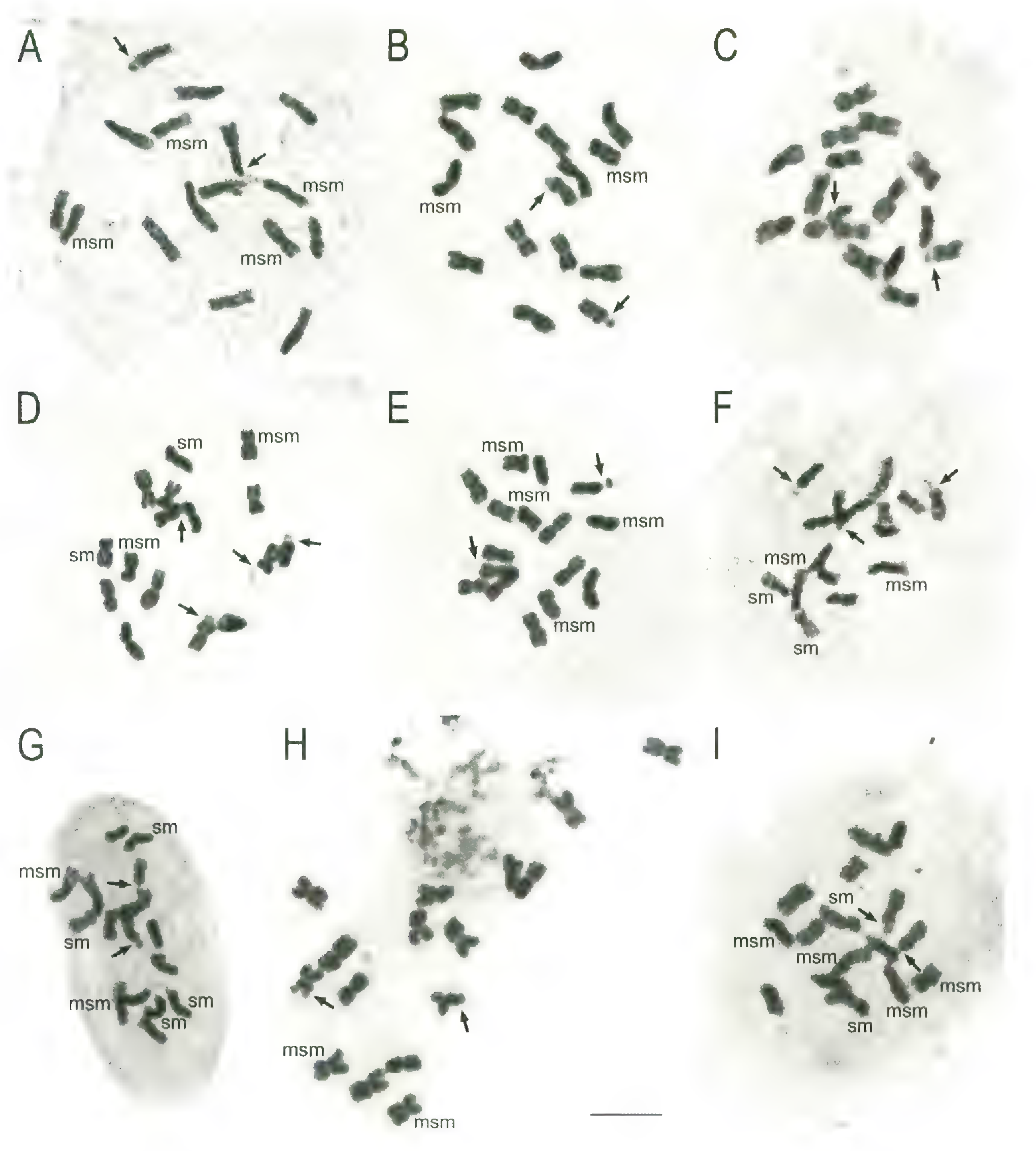

Figure 2. Somatic metaphases of *Nierembergia* species with $2n = 16$. —A. *N. calycina*. —B. *N. pulchella* var. *pulchella*. —C. *N. pulchella* var. *macrocalyx*. —D. *N. graveolens*. —E. *N. linariifolia* var. *pinifolioides*. —F. *N. repens*. —G. *N. rivularis*. —H. *N. tucumanensis*. —I. *N. veitchii*. Arrows (↑) indicate nucleolar organizing regions (NORs). Less frequent chromosome types are indicated: *msm*, meta-submetacentric; *sm*, submetacentric. Scale bar = 5 µm is the same for all figures.

chromosome number changes in the series of Nicotianeae in the context of the family. The pivotal position of $x = 11$ is noteworthy; thus, this number could represent a plesiomorphic state in Solanaceae. This basic number is dominant in the basal branching of the family (subfam. Salpiglossoideae (Benth.) Hunz. and tribe Browallieae), except for the earliest diverging lineages of *Schizanthus* Ruiz & Pav. (subfam. Schizanthoideae (Miers) Hunz.) and *Schwenckia* (subfam. Schwenckieae), both genera displaying $x = 10$, which may be derived by a decrease in chromosome number. In addition, $x = 11$ is the number of the suspected primitive Benthamielleae (Hunz.) Hunz. (Cestroideae) (Moscone, 1989a; Hunziker, 2001), a taxon not included in the molecular studies by Olmstead & Palmer (1992) and Olmstead et al. (1999). Reduction in chromosome number could also account for the appearance of $x = 8$ in all Cestreae, whose members are in a group sister to Browallieae, where $x = 11$ is typical (see Olmstead et al., 1999: figs. 3, 4).

Among the Nicotianeae, chromosomal reduction is also suggested by the presence of $x = 9$ in *Fabiana* and *Petunia*, both taxa forming a sister lineage to the tribe Franciseeae G. Don (Cestroideae) with $x = 11$, and of $x = 10$ in *Leptoglossis*, a genus molecularly not examined by Olmstead & Palmer (1992) and Olmstead et al. (1999). Further decrease from $x = 9$ to $x = 7$ in *Petunia* and to $x = 8$ in the monophyletic group composed of *Bouchetia*, *Hunzikeria*, and *Nierembergia* (see Olmstead et al., 1999: figs. 1, 2) could have occurred. Under this scheme, the rare $x = 9$ in the latter genus may be a dysploid increase from $x = 8$.

Finally, $x = 12$, almost universal in the monophyletic and derived cluster comprising *Nicotiana*, Solanoideae Schltdl., and Juanulloideae (Hunz.) Hunz. (Olmstead & Palmer, 1992; Olmstead et al., 1999), could have arisen by an increase from $x = 11$. Particularly in *Nicotiana*, all other basic numbers should be derived from $x = 12$, because they are restricted to sections *Suaveolentes* and *Alatae*, with the former group being a species assemblage geographically isolated and most probably derived (Goodspeed, 1954; Aoki & Ito, 2000). Additional chromosome and molecular data are needed to test these speculations.

POLYPLOIDY

All Nicotianeae species studied are diploids, although *Nierembergia rigida* and *N. aristata* also show tetraploid and hexaploid cytotypes, respectively. The presence of infraspecific polyploidy in the former species was unknown, while in the latter, Di Fulvio (1984) has cited the same levels of ploidy as reported here. Although polyploidy has played a significant role in the evolution of *Nicotiana*, where many species are amphidiploids (Chase et al., 2003), it does not appear to be relevant in the remaining genera of Nicotianeae as so far reported just for *Petunia spathulata* L. B. Sm. & Downs ($n = 18$; Stehmann et al., 1997) and *Hunzikeria texana* (Torr.) D'Arcy ($n = 16$; Whalen, 1979), in addition to the aforementioned species of *Nierembergia*. In the case of the hexaploid accession *Acosta et al. 32* (CORD) of *Nierembergia aristata*, the presence of multivalent configurations (particularly hexavalents) in meiosis suggests an autopolyploid origin. Concerning the polyploid samples *Subils et al. 4215* (CORD) of *N. aristata* and *Cocucci et al. 943* (CORD) of *N. rigida*, although they show a smaller number of NORs than expected (just one pair), they could have arisen by autopolyploidy, as suggested by Di Fulvio (1984) for another polyploid accession of the first species. The increased size of plant organs and structures in those populations compared to diploid counterparts in both species, and also reported previously for *N. aristata* (Di Fulvio, 1984), corresponds to the gigas phenotype more frequently present in autopolyploids than in allopolyploids (Singh, 1993). Further karyotypic and meiotic analyses of the polyploid samples here studied are required to cast light on their origin.

Both *Nierembergia* species with polyploid cytotypes have wide geographical distributions, although the hexaploid accessions of *N. aristata* in particular grow in flooded regions, while the tetraploid population of *N. rigida* occur in hilly habitats modified by man. The capability of polyploids to tolerate harsh ecological conditions and colonize disturbed habitats is well known (Stebbins, 1966, 1980). In particular, the population *Acosta et al. 32* of *N. aristata* seems to be of recent origin, because its ecological niche, at the border of a lake, arose about 100 years ago with the building of a dam in the region (Di Fulvio, 1984). The derived condition of polyploidy in *Nierembergia* is suggested by the fact that diploid complements are widespread in the genus.

SYSTEMATICS AND KARYOTYPE EVOLUTION

The results of the present work suggest that the species of *Nierembergia* can be grouped according to their karyotype features. Among the entities with $2n = 16$, two groups are distinguished. One of them, group I, is composed of *N. browallioides*, *N. calycina*, *N. linariifolia*, *N. pulchella*, and *N. tucumanensis*, with comparatively large chromosomes and more symmetrical karyotypes, composed only of *m* pairs or mainly of *m* and one to two *msm* pairs. It should be noted that *N. calycina* is the only species with NORs on long chromosome arms, perhaps as a result of an inversion; thus, it stands apart in group I. The other group (II) consists of *N. aristata*, *N. graveolens*, *N. repens*, *N. rigida*, *N. rivularis*, and *N. veitchii*, with smaller chromosomes and more asymmetrical complements, where one to two *sm* in addition to one to two *msm* pairs or three *msm* pairs are present. Finally, the species having $2n = 18$, *N. ericoides* and *N. scoparia*, form a third group (III), characterized by a small *st* chromosome pair. This grouping is supported by external morphology, reproductive biology, habit, and habitat affinities. The taxa included in groups I and III are small shrubs (except *N. calycina*, which is an herb), usually of mountain habitats, with single pollen grains and a semilunar stigma. On the other hand, group II is composed of rhizomatous, stoloniferous or tuber-bearing, and creeping herbs, except for the shrubby *N. graveolens*. These taxa grow in lowland habitats and are characterized by aggregates of pollen grains (except *N. graveolens* with single pollen grains) and a capitate stigma (Di Fulvio, 1976b; Cocucci & Hunziker, 1995).

In group III, $2n = 18$ seems to be a derived condition, because $2n = 16$ is present in most *Nierembergia* species and also in the related genus

Bouchetia. This variation in basic chromosome number could have arisen by a centric fission event during species diversification as has been postulated in other Solanaceae (Moscone et al., 1993). This hypothesis is supported by the presence of an additional small *st* chromosome pair in the taxa with $2n = 18$. On the other hand, group II, with morphological specializations such as much reduced branching, more pronounced zygomorphy (in part), and increased karyotype asymmetry, appears to have an advanced status relative to group I (Cocucci & Hunziker, 1995).

The similar karyotypes in the shrubby species of *Fabiana* studied is striking because the plants are morphologically very different from each other, i.e., *F. imbricata*, tall with comparatively imbricate leaves and conspicuous flowers, as well as *F. densa* and *F. denudata*, both comparatively small, but the former with a dense foliage of linear leaves and the latter nearly without leaves (Barboza & Hunziker, 1993). In *Petunia*, both species studied differ in chromosome number but not in karyotype asymmetry. In particular, *P. axillaris* has a similar karyotype to *P. hybrida* (Hook.) Vilm., another member of *Petunia* sensu Wijsman (1990) (a group with $x = 7$). On the other hand, the chromosome complement of *P. patagonica* does not differ from that of *P. linearis* (Hook.) Paxt., a species from the group with $x = 9$, which is now considered as *Calibrachoa* (Wijsman et al., 1983; Maizonnier, 1984). Chromosome features together with morphological traits, such as the corolla not entirely single colored and calyx lobes pentafid and not deeply incised, indicate that *P. patagonica* should be included in the *Calibrachoa* group (Wijsman & de Jong, 1985; Figueroa Romero, 1993; Mishiba et al., 2000). In general, *Petunia* sensu Wijsman displays advanced morphological features in comparison to *Calibrachoa* and the related genus *Fabiana*, as it comprises annual herbs without woody stems and brachyblasts (Barboza & Hunziker, 1993; Kulcheski et al., 2006).

The present results demonstrate that some genera of Nicotianeae, i.e., *Fabiana*, *Nierembergia*, and *Petunia*, display general karyotype patterns where uniformity in gross chromosome morphology (i.e., similar chromosome shape between taxa) is maintained even after changes in chromosome number. This occurs in several sections of *Nicotiana* (Goodspeed, 1954). Species-wide karyotypic orthoselection concerning both chromosome number and morphology has been observed in other genera of Solanaceae (e.g., *Cestrum*, *Capsicum* L., and *Solanum* L.) and other plant groups including an entire angiosperm family, as in the case of the Aloaceae (Moscone, 1989b; Bernardello & Anderson, 1990; Moscone et al., 1993; Brandham & Doherty, 1998).

Karyotypic data suggest that *Bouchetia*, with $x = 8$ and a rather symmetrical karyotype in the case of *B. anomala*, is closely related to *Nierembergia*. On the other hand, *Leptoglossis*, *Petunia*, and *Fabiana*, without $x = 8$ and with more asymmetrical complements except in the latter genus, appear more distant, in agreement with the suprageneric classification proposed by Hunziker (2001). Finally, the basic chromosome number recorded for *Hunzikeria* ($x = 8$) indicates affinity to *Bouchetia* and *Nierembergia* and a more distant relationship to *Leptoglossis*, in disagreement with Hunziker's (2001) system.

Literature Cited

Aoki, S. & M. Ito. 2000. Molecular phylogeny of *Nicotiana* (Solanaceae) based on the nucleotide sequence of the *matK* gene. Pl. Biol. (Stuttgart) 2: 316–324.

Badr, A., S. F. Khalifa, A. I. Aboel-Atta & M. M. Abou-El-Enain. 1997. Chromosomal criteria and taxonomic relationships in the Solanaceae. Cytologia 62: 103–113.

Barboza, G. E. & A. T. Hunziker. 1993. Estudios en Solanaceae XXXIV. Revisión taxonómica de *Fabiana*. Kurtziana 22: 109–153.

Bernardello, L. M. & G. J. Anderson. 1990. Karyotypic studies in *Solanum* section *Basarthrum* (Solanaceae). Amer. J. Bot. 77: 420–431.

Bowen, C. C. 1956. Freezing by liquid carbon dioxide in making slides permanent. Stain Technol. 31: 87–90.

Bradley, M. V. 1948. A method for making aceto-carmine squashes permanent without removal of the cover slip. Stain Technol. 23: 41–44.

Brandham, P. E. & M.-J. Doherty. 1998. Genome size variation in the Aloaceae, an angiosperm family displaying karyotypic orthoselection. Ann. Bot. (Oxford) 82 (Suppl. A): 67–73.

Chase, M. W., S. Knapp, A. V. Cox, J. J. Clarkson, Y. Butsko, J. Joseph, V. Savolainen & A. S. Parokonny. 2003. Molecular systematics, GISH and the origin of hybrid taxa in *Nicotiana* (Solanaceae). Ann. Bot. (Oxford) 92: 107–127.

Chiarini, F. E. 2003. Los cromosomas somáticos de *Schwenckia americana* (Solanaceae). Bol. Soc. Argent. Bot. 38: 325–327.

Cocucci, A. A. 1991. Pollination biology of *Nierembergia* (Solanaceae). Pl. Syst. Evol. 174: 17–35.

——— & A. T. Hunziker. 1995. *Nierembergia*. Pp. 3–14 *in* A. T. Hunziker (editor), Flora Fanerogámica Argentina, 256 Solanaceae, parte 1: Subtr. VII b. Nierembergiinae. Fasc. 15. Proflora (CONICET), Córdoba.

Cosa de Gastiazoro, M. T. 1989. Multiplicación vegetativa en Solanaceae: *Nierembergia stricta*, *N. aristata*, *Bouchetia anomala* y *Leptoglossis linifolia*. Kurtziana 20: 147–167.

D'Arcy, W. G. 1991. The Solanaceae since 1976, with a review of its biogeography. Pp. 75–137 *in* J. G. Hawkes, R. N. Lester, M. Nee & N. Estrada (editors), Solanaceae III: Taxonomy, Chemistry, Evolution. Royal Botanic Gardens, Kew.

Desai, S. R. 1970. Chromosome numbers and morphology in three Solanaceae. Phyton (Buenos Aires): 199–200.

Di Fulvio, T. E. 1976a. Recuentos cromosómicos en *Nierembergia* (Solanaceae). Kurtziana 9: 141–142.

———. 1976b. Sobre el polen de *Nierembergia* (Solanaceae). Kurtziana 9: 87–91.

———. 1978. Cromosomas gaméticos de *Nierembergia aristata* y *Bouchetia anomala* (Solanaceae). Kurtziana 11: 118.

———. 1984. Poliploidía en *Nierembergia* (Solanaceae). Kurtziana 17: 25–30.

Diers, L. 1961. Der Anteil an Polyploiden in den Vegetationsgürteln der Westkordillere Perus. Z. Bot. 49: 437–488.

Ferguson, M. C. 1924. Preliminary announcement of a cytological and a genetical study of *Petunia*. Anat. Rec. 29: 137. (Not seen; data from Goodspeed, 1954.).

Figueroa Romero, M. R. 1993. El Género *Petunia* (Solanaceae), Morfología y Taxonomía. Ph. D. Thesis, University of Tucumán, Argentina.

———. 1999. *Petunia*. Pp. 1084–1086 *in* F. O. Zuloaga & O. Morrone (editors), Catálogo de las Plantas Vasculares de la República Argentina II. Monogr. Syst. Bot. Missouri Bot. Gard. 74.

Gil, R. R., L.-Z. Lin, H.-B. Chai, J. M. Pezzuto & G. A. Cordell. 1995. Cardenolides from *Nierembergia aristata*. J. Nat. Prod. (Lloydia) 58: 848–856.

Godo, T., O. Tsujii, K. Ishikawa & M. Mii. 1997. Fertile transgenic plants of *Nierembergia scoparia* Sendtner obtained by a mikimopine type strain of *Agrobacterium rhizogenes*. Sci. Hort. 68: 101–111.

Goodspeed, T. H. 1933. Chromosome number and morphology in *Nicotiana* VI. Chromosome numbers of forty species. Proc. Natl. Acad. Sci. U.S.A. 19: 649–653.

———. 1954. The Genus *Nicotiana*. Chron. Bot. 16: 1–536.

Hunziker, A. T. 2001. Genera Solanacearum. The Genera of Solanaceae Illustrated, Arranged According to a New System. Gantner Verlag, Liechtenstein.

——— & R. Subils. 1979. *Salpiglossis*, *Leptoglossis* and *Reyesia* (Solanaceae), a synoptical survey. Bot. Mus. Leafl. 27: 1–43.

Infostat Group. 2002. INFOSTAT versión 1.1. Facultad de Ciencas Agropecuarias, Universidad Nacional de Córdoba. Editorial Brujas, Argentina.

Japan Tobacco. 1994. The Genus *Nicotiana* Illustrated. Japan Tobacco, Tokyo.

Jong, K. 1997. Laboratory Manual of Plant Cytological Techniques. Royal Botanic Garden, Edinburgh.

Jussieu, A. L. 1803. Sur le *Petunia*, genre nouveau de la famille des plantes solanées. Ann. Mus. Natl. Hist. Nat. 2: 214–216.

Kulcheski, F. R., V. C. Muschner, A. P. Lorenz-Lemke, J. R. Stehmann, S. L. Bonatto, F. M. Salzano & L. B. Freitas. 2006. Molecular phylogenetic analysis of *Petunia* Juss. (Solanaceae). Genetica 126: 3–14.

Levan, A., K. Fredga & A. A. Sandberg. 1964. Nomenclature for centromeric position on chromosomes. Hereditas 52: 201–220.

Maizonnier, D. 1984. Cytology. Pp. 21–33 *in* H. C. Sink (editor), *Petunia*. Springer-Verlag, Berlin.

Millán, R. 1941. Revisión de las especies de *Nierembergia* (Solanaceae). Darwiniana 5: 487–547.

Mishiba, K.-I., T. Ando, M. Mii, H. Watanabe, H. Kokubun, G. Hashimoto & E. Marchesi. 2000. Nuclear DNA content as an index character discriminating taxa in the genus *Petunia* sensu Jussieu (Solanaceae). Ann. Bot. (Oxford) 85: 665–673.

Moscone, E. A. 1989a. Karyotype analyses in three Patagonian and S. Andean endemic genera of Nicotianeae (Solanaceae). Pl. Syst. Evol. 166: 31–39.

———. 1989b. Estudios Citotaxonómicos en las Tribus Solaneae y Nicotianeae (Solanaceae) de América del Sur. Ph.D. Thesis, University of Córdoba, Argentina.

———. 1992. Estudios de cromosomas meióticos en Solanaceae de Argentina. Darwiniana 31: 261–297.

———, M. Lambrou, A. T. Hunziker & F. Ehrendorfer. 1993. Giemsa C-banded karyotypes in *Capsicum* (Solanaceae). Pl. Syst. Evol. 186: 213–229.

Olmstead, R. G. & J. D. Palmer. 1992. A chloroplast DNA phylogeny of the Solanaceae: Subfamilial relationships and character evolution. Ann. Missouri Bot. Gard. 79: 346–360.

———, J. A. Sweere, R. E. Spangler, L. Bohs & J. D. Palmer. 1999. Phylogeny and provisional classification of the Solanaceae based on chloroplast DNA. Pp. 111–137 *in* M. Nee, D. E. Symon, R. N. Lester & J. P. Jessop (editors), Solanaceae IV: Advances in Biology and Utilization. Royal Botanic Gardens, Kew.

Rangaswamy, N. S. & K. R. Shivanna. 1967. Induction of gamete compatibility and seed formation in axenic cultures of a diploid self-incompatible species of *Petunia*. Nature 216: 937–939.

Ratera, E. L. 1952. Número de cromosomas de algunas Solanáceas argentinas (Tercera contribución). Revista Fac. Agron. Veterin. 13: 280–284.

———. 1969. Estudios cariológicos en Solanáceas. II. Revista Inst. Munic. Bot. 3: 59–66.

Raven, P. H. 1975. The bases of angiosperm phylogeny: Cytology. Ann. Missouri Bot. Gard. 62: 724–764.

Schlarbaum, S. E. & T. Tsuchiya. 1984. The chromosomes of *Cunninghamia konishii*, *C. lanceolata*, and *Taiwania cryptomerioides* (Taxodiaceae). Pl. Syst. Evol. 145: 169–181.

Shizukawa, Y. & M. Mii. 1997. A simple and efficient plant regeneration system for leaf protoplasts of *Nierembergia repens* by inducing single shoots on the microcolonies. Pl. Cell. Rep. 16: 545–549.

Singh, R. J. 1993. Plant Cytogenetics. CRC Press, Boca Raton.

Sink, K. C. & J. B. Power. 1978. Incongruity of interspecific and intergeneric crosses involving *Nicotiana* and *Petunia* species that exhibit potential for somatic hybridization. Euphytica 27: 725–730.

Snow, R. 1963. Alcoholic hydrochloric acid-carmine as a stain for chromosomes in squash preparations. Stain Technol. 38: 9–13.

Sokal, R. R. & F. J. Rohlf. 995. Biometry. 3rd ed. Freeman, New York.

Stebbins, G. L. 1966. Chromosomal variation and evolution. Science 152: 1463–1469.

———. 1980. Polyploidy in plants: Unsolved problems and prospects. Pp. 495–520 *in* W. H. Lewis (editor), Polyploidy. Biological Relevance. Plenum Press, New York.

Steere, W. C. 1932. Chromosome behavior in triploid *Petunia* hybrids. Amer. J. Bot. 19: 340–357.

Stehmann, J. R., J. A. H. Dutilh, J. Semir & E. R. Forni-Martins. 1997. Solanaceae. P. 24 *in* C. A. Stace (editor), IOPB Chromosome Data 11. Int. Organ. Pl. Biosyst. Newslett. 26/27.

Subils, R. 1979. Cromosomas gaméticos de *Leptoglossis linifolia* (Solanaceae). Lorentzia 3: 19–20.

Sullivan, T. D. 1947. Somatic chromosomes of pedigreed hybrid *Petunia*. Bull. Torrey Bot. Club 74: 453–475.

Watanabe, H., T. Ando, S. Iida, A. Suzuki, K. Buto, T. Tukamoto, G. Hashimoto & E. Marchesi. 1996. Cross compatibility of *Petunia* cultivars and *P. axillaris* with native taxa of *Petunia* in relation to their chromosome number. J. Hort. Assoc. Japan 65: 625–634.

Whalen, M. D. 1979. Solanaceae. P. 276 *in* A. Löve (editor), IOPB Chromosome Number Reports LXIII. Taxon 28.

Wijsman, H. J. W. 1990. On the inter-relationships of certain species of *Petunia* VI. New names for the species of *Calibrachoa* formerly included into *Petunia* (Solanaceae). Acta Bot. Neerl. 39: 101–102.

——— & J. H. de Jong. 1985. On the interrelationships of certain species of *Petunia* IV. Hybridization between *P. linearis* and *P. calycina* and nomenclatorial consequences in the *Petunia* group. Acta Bot. Neerl. 34: 337–349.

———, ——— & T. M. Pedersen. 1983. On the interrelationships of certain species of *Petunia* III. The position of *P. linearis* and *P. calycina*. Acta Bot. Neerl. 32: 323–332.